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PRINCIPAL INVESTIGATOR: Dr. Vikhyat Bebarta

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Dr. Vikhyat Bebarta

E-Mail: vikhyat.bebarta@us.af.mil

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14. ABSTRACT

Non-medical use and abuse of prescription opioids is a growing problem in both the civilian and military communities. Current technologies for detecting hydrocodone use are limited. Standard drug screens do not detect hydrocodone. In order to detect the use of hydrocodone and prescription opioids for nontherapeutic purposes, it is vital to establish the excretion profile of these drugs. Currently there is no data available describing blood, urine and oral fluid profiles following administration of a 10 mg dose of hydrocodone. We will measure proteomes and metabolites in blood, urine and oral fluid samples after hydrocodone exposure. We are exposing healthy volunteers (n = 30) to 10 mg pure hydrocodone under controlled conditions and collecting blood, oral fluid, and urine at defined intervals up to 7 days. We will include 2 subjects for control in the study giving a total of 32 participants.

15. SUBJECT TERMS

Proteomic, drug abuse, opioids, metabolites, and pharmacokinetic

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INTRODUCTION:

The purpose of the overall protocol is to study the metabolism and protein expression in the urine and blood of human subjects administered hydrocodone. An opioid is prescribed as a pain medication to the patient to minimize pain. Hydrocodone will be administered to healthy volunteers. Urine and blood will be collected prior to and following administration of the drug. The three separate biofluids will be analyzed for drug and metabolites and for changes in protein expression. Changes in protein expression will provide a general understanding of opioid exposure in future studies relating to opioid abuse.

BODY:

Phase 1 – Single Dose Administration

1. **Institutional Review Board (IRB) application**
    - IRB approved protocol, ICD, and HIPAA received in Q1. Annual Review submitted to IRB and study approved for another year, expires May 2013.
    - We have received IRB approval to increase enrollment from 32 to forty (40) Subjects.
        - We are replacing four (4) Subjects who vomited on Day 1, two (2) Subjects we could not obtain blood from on Day 1, and two (2) subjects that signed the ICD but were excluded because they did not meet Inclusion/Exclusion criteria. See Table 1

2. **Research Nurse coordinator**
    - Research Nurse was hired in Q1, employed via the Geneva Foundation.

3. **Lab technician**
    - Lab technician was hired in Q1, employed via the Geneva Foundation.

Phase 2 – Patient recruitment

4. **Drug Administration, biofluid sampling and PK Analysis**
    - Thirty-five (35) Subjects have been enrolled. See Table 1 below
    - Recruitment and enrollment is on target.
    - We have received IRB approval to replace the four (4) Subjects who vomited on Day 1 after receiving study drug (Subjects 9, 10, 19, and 26).
    - We have received IRB approval to replace Subjects 29 and 31. These individuals were withdrawn from the study on Day 1 because we were unable to withdraw blood from them.
    - Last Subject should be completed in the 1st Quarter of 2012.
        - Urine is being collected for up to 5 days following administration of hydrocodone.
        - Blood is collected at specified time points throughout the first day then at 24, 48, 96, and 168 hour post dose.
        - Samples are being stored refrigerated or frozen until analysis.
        - Liquid chromatography-mass spectrometry (LC-MS/MS) method validations for analysis of hydrocodone and metabolites in urine and plasma using UCT Excel I solid phase extraction columns have been completed.
        - Analysis of hydrocodone and metabolites in Subject plasma samples by LC/MS/MS has been completed for Subjects 1-34.
        - Subject urine samples are stored frozen and will be analyzed following analysis of plasma samples.

Table 1

Subject Number	Informed Consent and HIPAA Signed	Randomization/ Enrollment Date	Number of Blood samples collected	Number of Urine samples collected	Number of Oral Fluids collected	Completion or Discontinuation Date	Number of Visits Attended (should be 6, M,T,W,TH,F,M)	SAE	Reason for Discontinuation, if applicable
01	Yes	14 March 2011	20	29	45	21 March 2011	6	0	Completed study
02	Yes	14 March 2011	19	36	44	21 March 2011	6	0	Completed study
03	Yes	04 April 2011	20	31	33	11 April 2011	6	0	Completed study
04	Yes	18 April 2011	20	29	47	25 April 2011	6	0	Completed study
05	Yes	18 April 2011	20	24	42	25 April 2011	6	0	Completed study
06	Yes	25 April 2011	19	31	42	02 May 2011	6	0	Completed study
07	Yes	25 April 2011	20	34	47	02 May 2011	6	0	Completed study
08	Yes	09 May 2011	20	28	41	16 May 2011	6	0	Completed study
09	Yes	06 June 2011	21	24	40	13 June 2011	6	0	Completed study Replace- vomited
10	Yes	13 June 2011	20	28	43	20 June 2011	6	0	Completed study Replace- vomited
11	Yes	13 June 2011	20	34	50	20 June 2011	6	0	Completed study
12	Yes	20 June 2011	20	35	47	27 June 2011	6	0	Completed study
13	Yes	20 June 2011	20	30	42	27 June 2011	6	0	Completed study
14	Yes	20 June 2011	20	29	41	27 June 2011	6	0	Completed study
15	Yes	25 July 2011	20	30	42	01 Aug 2011	6	0	Completed study
16	Yes	01 Aug. 2011	20	23	38	08 Aug. 2011	6	0	Completed study
17	Yes	01 Aug. 2011	20	35	45	08 Aug 2011	6	0	Completed study
18	Yes	08 Aug 2011	20	24	39	15 Aug 2011	6	0	Completed study
19	Yes	15 Aug 2011	20	31	43	22 Aug 2011	6	0	Completed study Replace- vomited
20	Yes	15 Aug 2011	20	26	39	22 Aug 2011	6	0	Completed study
21	Yes	12 Sep 2011	20	31	43	19 Sep 2011	6	0	Completed study
22	Yes	12 Sep 2011	20	44	53	19 Sep 2011	6	0	Completed study
23	Yes	26 Sep 2011	20	18	32	03 Oct 2011	6	0	Completed study
24	Yes	26 Sep 2011	16 No collection after Day 2	37	51	03 Oct 2011	6	0	Completed study
25	Yes	26 Sep 2011	20	23	37	03 Oct 2011	6	0	Completed study

26	Yes	24 Oct 2011	20	24	39	31 Oct 2011	6	0	Completed study Replace- vomited
27	Yes	14 Nov 2011	20			21 Nov 2011	6	0	Completed study
28	Yes	14 Nov 2011	19 No collection Day 1 Specimen # 15	21	37	21 Nov 2011	6	0	Completed study
29	Yes	12 Dec 2011	0	0	0	12 Dec 2011	1	0	Withdrew on Day 1 Unable to withdraw blood from angiocath after baseline Drug administered, observed for 5 hours.
30	Yes	12 Dec 2011	20	27	40	19 Dec 2011	6	0	Completed study
31	Yes	12 Dec 2011	0	0	0	12 Dec 2011	1	0	Withdrew on Day 1 Unable to withdraw blood from angiocath Drug not administered
32	Yes	23 Jan 2012	20	32	46	30 Jan 2012	6	0	Completed study
33	Yes	23 Jan 2012	20	24	35	30 Jan 2012	6	0	Completed study
34	Yes	06 Feb 2012	20	48	53	13 Feb 2012	6	0	Completed study
35	Yes	06 Feb 2012	20	36	44	13 Feb 2012	6	0	Completed study

5. Proteomic analyses

- Analysis for drug and metabolites in plasma is underway. Sample sets 1-12 have been completed.
- 0.5 mL of selected time points for Subjects 1-12 plasma samples have been shipped and received by PNNL.
    - Preliminary metabolic analysis of the kinetics of hydrocodone and hydromorphone levels in the plasma post-treatment guided time point selection for proteomic LC-MS/MS analysis. Selected time points (pre-treatment, 1, 2, 4, 8, and 48 hours post-treatment) captured each subject's baseline protein levels, the peak of drug levels (usually between the 2-8 hour time points), and a return to baseline levels upon metabolism of the drug.
- Pre-MS plasma sample processing at PNNL included depletion of high to moderately abundant plasma proteins using human IgY14 and IgY supermix immunoaffinity columns along with tryptic digestion and isolation of the subsequent peptides for LC-MS/MS analysis. Immunoaffinity depletion allows an increase in the dynamic range of detection and identification of less abundant proteins and potentially more subtle changes in the plasma proteome.
- All LC-MS analyses are complete. Total instrument analyses included: 12 subjects x 6 time points per subject x 2 technical duplicates = 144 datasets. Samples were analyzed on a hybrid high resolution and high mass accuracy LC-MS/MS platform (ThermoScientific LTQ Orbitrap Velos) which couples peptide identification (tandem MS data) with high resolution peak intensity data for quantification. The data has been processed via the AMT tag approach for label-free quantification of peptide abundance.

- Currently, the 144 datasets are being analyzed to identify peptides/proteins which show statistically significant similarities and/or differences across the plasma proteome, within individuals and across time points, in response to hydrocodone administration.

6. **Interpretation –** results of PK and proteomic analyses will be evaluated to determine a signature of hydrocodone use and to validate LC-MS/MS approach.
    - Mass spectrometric analyses results:
        - Total combined number of unique peptides identified (in all datasets): 12,907 peptides.
        - Total combined number of unique proteins identified (in all datasets): 1,074 proteins.
        - Number of unique proteins identified per subject (in at least one sample): range 570-867 proteins, average 688 proteins.
    - Preliminary data analysis:
        - Initial review of the data demonstrates a subset of plasma proteins which potentially appear to respond temporally following hydrocodone administration, through either increased or decreased abundance at specific time points (see Figure 1 for example). The first priority of data analysis will be to identify such proteins of interest. Similarities and differences in protein responses of interest between subjects will then be determined.

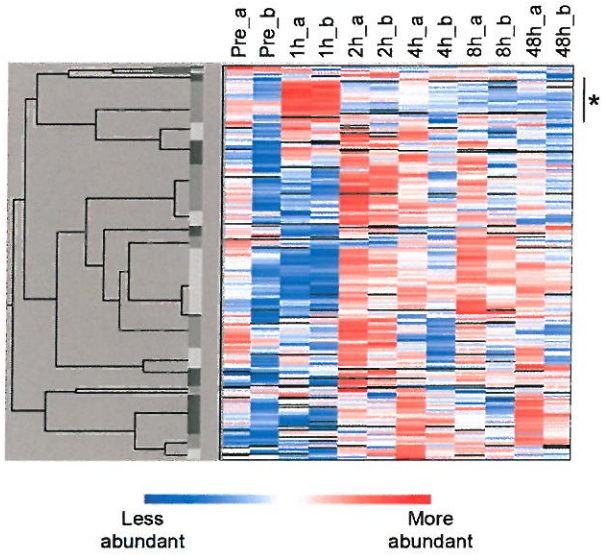

Figure 1. Heat map of protein abundance data (651 proteins) from Subject 7 representing duplicate mass spectral analyses of 6 time points. Preliminary analyses revealed temporal responses to hydrocodone treatment at specific time points (see proteins in the cluster marked with an asterisk showing marked increase at 1 hour post-treatment). Such protein trends which correlate temporally with hydrocodone metabolism are of primary interest.

Phase 3 –

7. **PNNL-Proteomics:** Data products will include all protein identifications produced during the project for each sample, a list of putative markers for opioid use through time, and preliminary biological interpretation through the form of a manuscript.

8. **WHMC-Pharmacokinetic evaluation:** Data products will include metabolism, excretion, and pharmacokinetic profiles for each subject set, and interpretation through the form of a manuscript.

9. **Manuscript preparation and results dissemination**
   No publications to date.

KEY RESEARCH ACCOMPLISHMENTS:

- Enrollment is on target.
- Preliminary data analysis is ongoing.
- Mass spectrometric analyses.
- Analysis for drug and metabolites in plasma is underway.

REPORTABLE OUTCOMES:

There have been no manuscripts, abstracts, presentations; patents and licenses applied for and/or issued.

CONCLUSION:

In conclusion, we plan to complete enrollment in the first quarter of 2012 and continue analysis.

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APPENDICES:
No appendices